

Research Article

## Influence of salicylic acid nano-formulation on expression of peroxidase (113-114) genes and peroxidase and phenylalanine ammonia lyase in wheat cultivar susceptible to *Heterodera filipjevi*

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**Abstract:** The effects of salicylic acid (SA) nano-formulation on expression of peroxidase (113-114) genes and peroxidase and phenylalanine ammonia lyase (PAL) were investigated in wheat cultivar (Bezostaya) susceptible to *Heterodera filipjevi*. The wheat roots and leaves were randomly divided into control group and groups exposed to 62.5, 125 and 250 µg/ml SA. A spectrophotometric analysis was carried out using root extracts from infected plants at 4, 7 and 11 days post inoculation with nematode (DAI) for peroxidase and PAL. The expression of peroxidase (113-114) genes was evaluated by Real time PCR analysis. Peroxidase activity was significantly increased in treatments exposed to 250 µg/ml of nanosalicylic acid at 11 DAI. Phenylalanine ammonia lyase activity was induced in the treatments exposed to 250 and 125 µg/ml nanosalicylic acid compared to the control at 4 and 7 DAI, respectively. Phenylalanine ammonia lyase activity was also increased in the treatments exposed to 62.5 and 250 µg/ml of nanosalicylic acid compared to the control at 7 DAI. The expression level of peroxidase 113-114 in wheat leaves was significantly raised at 4 DAI when 62.5 µg/ml of nanosalicylic acid was used. There was also a significant difference between expression levels of peroxidase 113-114 genes at applications of 125 and 250 µg/ml of SA in comparison with the control at 4 and 7 DAI, a significant decrease was revealed in the gene expression in treatments exposed to 62.5, 125 and 250 µg/ml of nanosalicylic acid compared to the control at 11 DAI. It was concluded that higher concentrations of nanosalicylic acid have a potential effect on peroxidase and PAL activities in wheat infected by *H. filipjevi*. High concentration of nanosalicylic acid has inhibitory effects on the expression level of peroxidase gene.

**Keywords:** Cereal Cyst Nematode, PAL, Peroxidase, SA

### Introduction

Cereals such as wheat, barley and oats are among the major staple crops with economic

importance worldwide (Kaur *et al.*, 2014). Wheat *Triticum aestivum* L. is one of the most important cultivated crops in Iran and is cultivated in all parts of the country under both irrigated and rain-fed conditions. The area under wheat cultivation was 5.7 million ha comprising 39% and 60% irrigated and non-irrigated, respectively in 2015 (Anonymous, 2016) and data have shown that 0.8 million ton

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(~8% of Iran's wheat production in 2014-2015) of wheat grain might be produced per year by allocating one million ha of the unused lands from the medium suitability class to rainfed wheat cropping (Mesgaran 2017). Cereal cyst nematodes, CCNs are globally known as the parasites of cereals and grasses, they limit the production of small grain cereals by invading the cereal host plants (Smiley *et al.*, 2017). The nematodes form specific large multinucleate cells resulted from breakdown of cell walls and fusion of adjacent protoplasts called syncytia close to the vascular system of the root (Zhang *et al.*, 2017). The crop losses caused by this group of nematodes have been reported from 20% in Pakistan. 50% both in Australia and Turkey up to 90% in Saudi Arabia (Riley *et al.*, 2009). The effect of *H. filipjevi* on wheat and barley were evaluated under microplot and field conditions in different regions of Iran in recent years, viz Markazi, Khuzestan and Isfahan Provinces (Hajihassani *et al.*, 2010; Ahmadi *et al.*, 2013; Karimipour Fard *et al.*, 2018). A more recent investigation proved the reduction of grain yield due to *H. filipjevi* from 20.4% up to 24.8% in three wheat cultivars in field conditions, meanwhile the reproduction factor was significantly reduced in plots by nematicide application (Karimipour Fard *et al.*, 2018). Nematode management is mostly performed through application of a combination of agricultural, chemical, biological methods and resistant cultivars (Timper 2014).

Plants have evolved various defense mechanisms to resist infection by pathogens (War *et al.*, 2012). The phytohormone salicylic acid is an important signal molecule produced by a wide range of prokaryotic and eukaryotic organisms including plants (Lareen *et al.*, 2016). Plant disease resistance is associated with high expression of defense genes and the accumulation of salicylic acid in the inoculated leaves. Plants often respond to the environmental stresses with increasing SA level (Loake and Grant, 2007; Oka and Cohen, 2001). Salicylic acid also regulates the

activities of different enzymes such as peroxidase, polyphenol oxidase, superoxide dismutase and PAL which are the major components of induced plant defense against biotic and abiotic stresses. These enzymes play a significant role in the defense of plants against pathogens by catalyzing cell wall lignification. The pathways and regulation of SA biosynthesis in plants may be more complicated than was thought earlier (Van Loon *et al.*, 2006; War *et al.*, 2011 b).

Studies have shown that Plant-parasitic nematodes cause considerable damages to the agricultural crops worldwide and may kill a plant and reduce its productivity through feeding on the plant (Zinovieva, 2014; Ladner *et al.*, 2008; Bird *et al.* 2015). There are also several reports indicating that SA is a key hormone in the plant defense which plays an important role in the local and systemic defense responses against various pathogens (Loake and Grant, 2007; Rabe *et al.*, 2013).

Salicylic acid is a part of the Mi-1-mediated defense response to the root-knot nematode in tomato. Spraying SA may reduce root galling and increase plant growth in tomato infected by root knot nematode, *Meloidogyne javanica* (Almaghrabi *et al.*, 2013). Phytochemical research have shown that  $\beta$ -aminobutyric acid reduces the number of *H. avenae* and *H. latipons* cysts on wheat and barley as plant resistance inducer and acetylsalicylic acid can reduce reproduction of *H. glycines* on the susceptible soybean cultivar (Oka and Cohen 2001). Recent studies have demonstrated that PAL activity has increased in response to different concentrations of SA at 5 days post inoculation (Ketabchi *et al.*, 2014). Another known function of peroxidases is generating reactive oxygen species (ROS) in SA defense response pathways (Almagro *et al.*, 2009). Experimental evidences also implicate class III peroxidases in plant defense response to pathogen/pest attacks, such as bacteria (Ahola-Iivarinen *et al.*, 2009), fungi (Chassot *et al.*, 2007), viruses (Periago *et al.*, 2006) insects (Little *et al.*, 2007) and cyst nematodes (Delibes *et al.*, 2009) which play pivotal roles in SA

defense response pathway by generating ROS (Jing *et al.*, 2011). In contrast, several evidences indicated that exposure to SA for 2 days after inoculation may reduce PAL activity (Ketabchi *et al.*, 2014).

A large amount of data has focused on the relation between SA and plants infected by nematodes, but the results are still conflicting in many aspects. The aim of this research was to investigate the effects of salicylic acid nano-formulation on expression of peroxidase (113, 114) and PAL in wheat plants susceptible to *H. filipjevi*.

## Materials and Methods

### Nematode preparation

Infested soil with *H. filipjevi* was collected from a wheat field in Hamadan province. The cysts were extracted by Fenwick-can technique (Fenwick, 1940). Cysts were picked using forceps, and surface sterilized in 0.5 percent NaOCl for 10 minutes and were rinsed several times in distilled water. The cysts were kept in the refrigerator at 4 °C and then were transferred to room temperature (10-15 °C) to enhance hatching. The freshly hatched nematode juveniles (J2) were collected in a glass beaker. Later, the nematode number per milliliter of nematode suspension was calibrated by taking average count (three aliquots) under the stereomicroscope.

### Preparation of wheat genotypes

The soil (in small tube) in which wheat were grown was inoculated with the second stage juveniles (J2) at a population of 500 J2 per plant according to Dababat (2012) with some modification. Wheat cultivar (Bezostaya) susceptible to *H. filipjevi* was used in this experiment. Seeds were germinated on wet paper and sown in standard small tubes (16 cm in height × 2.5cm in diameter) filled with a sterilized mixture of sand, field soil, and organic material (70:29:1 v/v) which were maintained at 25 ± 2 °C in a growth chamber with a 16-hr photoperiod of artificial light and relative humidity of 70% (± 5). After 7

days, *H. filipjevi* J2 were added into the soil around the plant crown. The experiment was carried out as a completely randomized design with nine treatments and three replicates

### Chemicals

Salicylic acid was obtained from Pasargad Novin Company (Iran) and its nano-formulations were fabricated with a surfactant-assisted ball milling process followed by a centrifugal separation. Standard solutions of different concentrations of formulated SA consisted of 62.5, 125 and 250µg/ml. Then the plants were sprayed using different concentration of nano-formulations of salicylic acid 5 days after seed germination.

### Plant harvest

The plants were harvested at 63 days post inoculation and their roots were washed under slow running tap water for 1-2 min to remove the soil particles. The roots were sprayed with a strong water jet to dislodge white females and brown cysts that were collected on the lower sieve and counted under a stereomicroscope. The cysts were extracted from soil by Fenwick Can method technique (Fenwick, 1940). The data were analysed using MSTATC Software data statistical analysis and the means were grouped based on the least significant difference test.

### Peroxidase activity assay

Peroxidase activity was assayed using the infected wheat roots at 4, 7 and 10 days post inoculation with *H. filipjevi* (Andres *et al.*, 2001; Delibes *et al.*, 2008; Simonetti *et al.*, 2010). Peroxidase activity was measured using Malik and Singh (1980) method. 0.5 Grams of infected plant root was extracted in 3ml of 0.1 M phosphate buffer at pH 7.0 by grinding root pieces in a pre-chilled mortar. The homogenate was then centrifuged at 18000g for 15min at 5 °C and the supernatant as enzyme source was stored on ice till the assay was carried out. The amount of 3ml of the buffer solution, 0.05ml guaiacol solution,

0.1ml enzyme extract and 0.03ml hydrogen peroxide solution were pipetted out in a cuvette. The mixture was well shaken and placed in the spectrophotometer. The time required for the mixture optical density to be increased by 0.1 ( $\Delta t$ ) at 436nm was recorded and used in the calculations. The enzyme specific activity units were calculated using the following formula:

$$g^{-1} f wt = [500/\Delta t] \times [1/1000] \times [TV/VU] \times [1/f wt], \text{ where;}$$

$\Delta t$  = time change in minute; TV = total volume of the extract (ml); VU = volume used (ml); f wt = weight of the fresh root tissue (g).

#### Assay of Phenylalanine ammonia-lyase activity

Phenylalanine ammonia lyase activity was assayed using the infected wheat roots at 4, 7 and 11 days post inoculation. Phenylalanine ammonia lyase was quantitatively determined at 290nm using a spectrophotometer following the Peltonen and Karjalainen (1995) method. Reaction mixture containing 2.5ml of 0.2% L-phenylalanine in 50mM Tris-HCl (pH 8.5), and 0.5ml of enzyme extract was incubated for 1 h at 40 °C after which, the optical density was recorded at 290nm. One unit of enzyme is defined as the amount of protein that catalyzed the appearance of 1 $\mu$ mol cinnamic acid/min at 30 °C.

#### Quantitative Real Time-PCR Analysis

To measure the expression of peroxidase genes (POX 113-114) in nematode infected wheat leaves at 4, 7 and 11 days post inoculation, total RNA was isolated using the TRIZOL reagent according to the manufacturer's instructions (Gene all, South Korea). Traditionally, RNA is quantified by measuring UV absorbance using a spectrophotometer (Thermo Fisher Scientific Inc., Waltham, MA, USA). Through measuring the optical density of RNA solution at wavelengths of 260 and 280 nm, it is possible to determine the concentration of solution as well as the presence of contaminants such as DNA, proteins and salts

in the sample. A pure RNA sample has a 260/280 ratio range of 1.80 to 2.00. Total RNA was reverse transcribed into cDNA using a transcriptor first strand cDNA synthesis kit (Applied Biosystems), and quantitative real time PCR was carried out by using a LightCycler-FastStart DNA master SYBR Green I Kit (Applied Biosystems) and LightCycler apparatus (Roche Diagnostics).

The RT-PCR for peroxidase genes (POX 113-114) was carried out using the specific primers (Table 1). Actin gene was used to normalize the relative expression for genes of interest and calculated by  $2^{\Delta\Delta CT}$  method and SYBR Green kit. The presence of the expected PCR products after quantitative real-time RT-PCR reactions was confirmed by an agarose gel electrophoresis.

**Table 1** Specific primers for amplifying Peroxidase113, POX114 and Actin genes.

Genes	Primers	bp
Peroxidase 113	ATTGACAAACGAGTTACTGCCTACTAG (forward)	27
	GATTTGCTGCTGCTCGTACA (reverse)	20
Peroxidase 114	CGGTGACACCAACATCAACACTG (forward)	23
	CAGGAGCCCTTTCTGTGACAG (reverse)	21
Actin	GAAGCTGCAGGTATCCATGAGACC (forward)	24
	AGGCAGTGATCTCCTTGCTCATC (reverse)	23

#### Statistical analysis

The data was expressed as mean  $\pm$  standard deviation (SD). One-way Analysis of Variance (ANOVA) and Turkey's post hoc-test were used to determine significant difference between groups ( $P < 0.05$ ) in SPSS 20 software.

#### Results

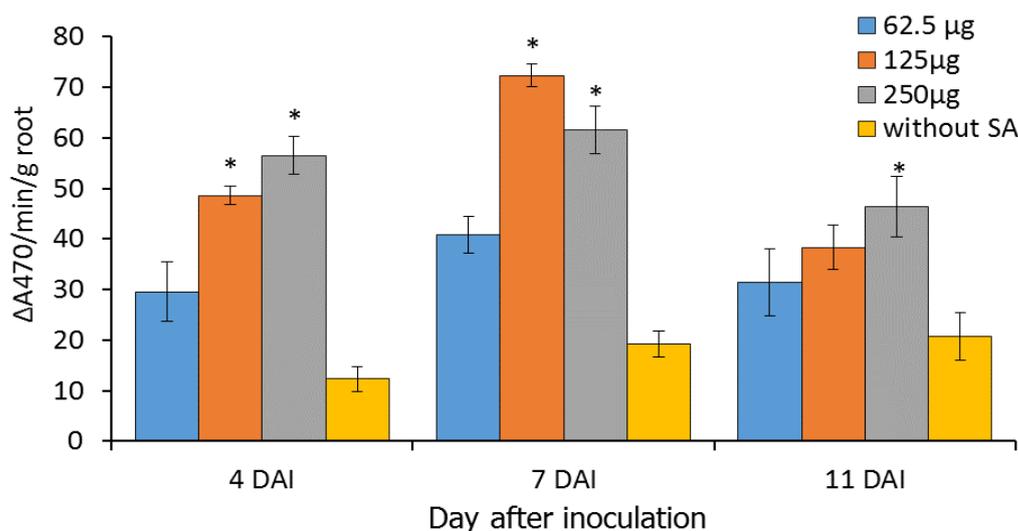
According to Fig. 1, there was no significant difference in peroxidase activity of wheat exposed to 62.5 $\mu$ g/ml of nanosalicylic acid compared to control (without salicylic acid) and no difference was detected between

peroxidase activity of wheat exposed to 250 $\mu$ g/ml of nanosalicylic acid in comparison with 125 $\mu$ g/ml of nanosalicylic acid at 4 and 7 days post inoculation (DAI) either. However, peroxidase activity was significantly increased in the treatments exposed to 125 and 250 $\mu$ g/ml of nanosalicylic acid compared to 62.5 $\mu$ g/ml of nanosalicylic acid and control at 4 and 7 DAI ( $P < 0.05$ ). There was also no statistically significant difference between the peroxidase activity of nanosalicylic acid at 62.5 and 125 $\mu$ g/ml compared with control at 11 DAI and no difference was found between peroxidase activity at 250 and 125 $\mu$ g/ml of nanosalicylic acid in comparison with 62.5 $\mu$ g/ml at 11 DAI. Although, peroxidase activity was increased considerably in treatments exposed to 250 $\mu$ g/ml of nanosalicylic acid at 11 DAI ( $P < 0.05$ ).

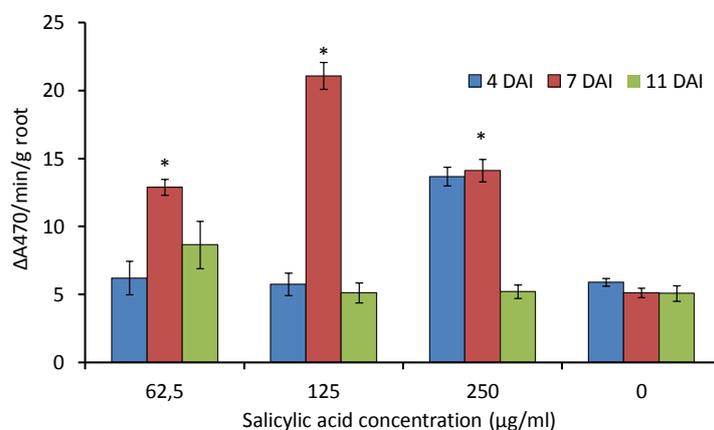
As shown in Fig. 2, Phenylalanine ammonia lyase activity increased in the treatment exposed to 250 $\mu$ g/ml of nanosalicylic acid compared to 62.5 and 125 $\mu$ g/ml of nanosalicylic acid and control at 4 DAI ( $P < 0.05$ ). No significant difference was detected between PAL activity of wheat exposed to 62.5 and 125 $\mu$ g/ml of nanosalicylic

acid in comparison with control at 4 DAI. However, there was a significant increase in PAL activity in treatments of 125 $\mu$ g/ml of nanosalicylic acid compared to the other levels and control at 7 DAI ( $P < 0.05$ ). The PAL activity substantially increased in the treatments exposed to 62.5 and 250 $\mu$ g/ml of nanosalicylic acid compared to control at 7 DAI ( $P < 0.05$ ) and no significant difference was observed between these treatments at 7 DAI. No significant difference was observed in the PAL activity for different concentrations of nanosalicylic acid in comparison with control at 11 DAI.

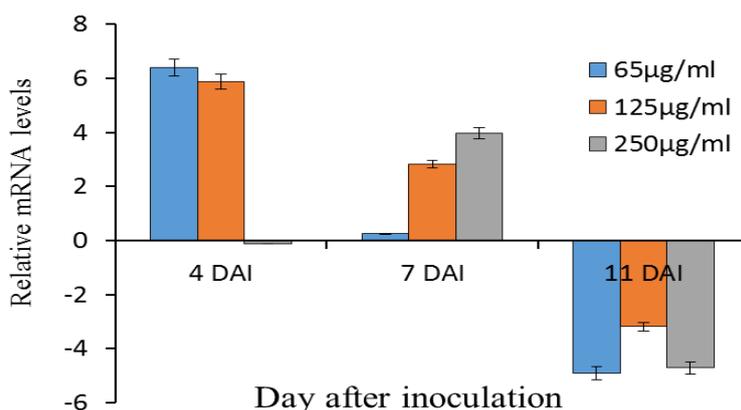
Figures 3 and 4 show expression levels of peroxidase 113 and peroxidase 114 genes in wheat exposed to 62.5, 125 and 250 $\mu$ g/ml of nanosalicylic acid and at 4, 7 and 11 DAI, respectively. According to figures 3 and 4 nanosalicylic acid at 62.5 $\mu$ g/ml raised significantly the expression level of peroxidase 113-114 in wheat leaves at 4 DAI and there was also slight increase in their expression level compared to the control at 7 DAI. Although there was a significant decrease between genes expression exposed to 62.5 $\mu$ g/ml of nanosalicylic acid compared to the control at 11 DAI.



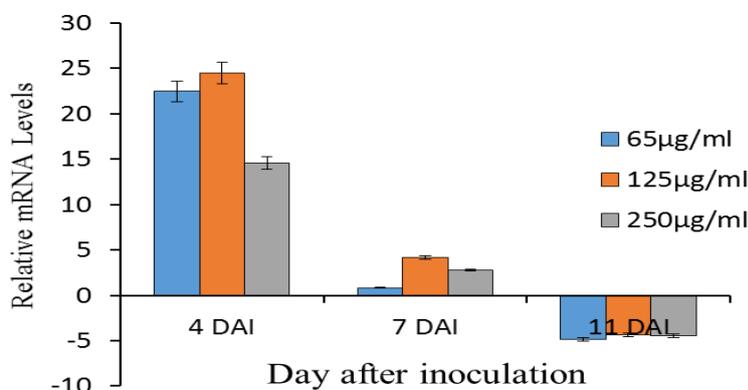
**Figure 1** Effect of different concentrations of nanosalicylic acid on peroxidase activity in wheat cultivar Bezostaya susceptible to *Heterodera filipjevi* at 4, 7 and 11 days post inoculation. (\*): Values are statistically significant at  $P < 0.05$  compared to control group.



**Figure 2** PAL activity of wheat cultivar Bezostaya susceptible to *Heterodera filipjevi* treated with different concentration of nano-salicylic acid at 4, 7 and 11 days after inoculation (DAI). (\*): Values are statistically significant at P < 0.05 compared to control group.



**Figure 3** Effect of different concentrations of nanosalicylic acid on expression of peroxidase 113 gene on wheat cultivar Bezostaya susceptible to *Heterodera filipjevi* at 4, 7 and 11 days after inoculation (DAI).



**Figure 4** Effect of different concentrations of nanosalicylic acid on expression of peroxidase 114 gene on wheat cultivar Bezostaya susceptible to *Heterodera filipjevi* at 4, 7 and 11 days after inoculation (DAI).

There was also significant increase between expression level of peroxidase 113-114 genes at 125µg/ml in comparison with control at 4 and 7 DAI and a significant reduction was observed in the expression level of peroxidase 113-114 exposed to 125µg/ml of nanosalicylic acid at 11 DAI.

Little reduction was found in the expression level of peroxidase 113 gene in 250µg/ml of nanosalicylic acid concentration in comparison with control at 4 DAI. Although, peroxidase 114 gene expression level significantly increased in the treatments with 250µg/ml of nanosalicylic acid at 4 DAI. There was also great increase in expression level of peroxidase 113-114 genes exposed to 250µg/ml of nanosalicylic acid compared with the control at 7 DAI. However, the expression levels of peroxidase 113-114 genes eased and considerably decreased in the treatment with 250µg/ml of nanosalicylic acid compared to control at 11 DAI.

According to table 2, there was significant decrease between the cyst number of wheat susceptible to nematode exposed to 62.5, 125 and 250µg/ml different concentrations of nano-salicylic acid compared to control (treated with nematode only).

**Table 2** Analysis of variance for the cyst number in wheat cultivar Bezostaya susceptible to *Heterodera filipjevi* in response to different concentrations of nano-salicylic acid (SA).

variable	Number of samples	Sum	Mean	SD	SE	df
control	4.00	49.00	12.25	2.50	0.93	3
62.5µg/ml SA	4.00	17.00	4.25*	0.50	0.93	3
125µg/ml SA	4.00	16.00	4.00*	0.82	0.93	3
250µg/ml SA	4.00	32.00	8.00*	2.58	0.93	3

Coefficient of Variation = 26.10%.

\*indicate significant differences at  $P < 0.05$  compared to control.

## Discussion

The results obtained from the greenhouse investigation proved that the different concentrations of salicylic acid cause a reduction in the cyst number of *H. filipjevi* in the susceptible wheat cultivar. In line with our investigation several reports indicate that SA induces plant immune system which can respond to various stresses and infections especially nematode infections (Asselbergh *et al.*, 2008; Rejeb *et al.*, 2014).

We have also shown that Spraying wheat leaves with nanoSA at high concentrations increases PAL activity at 4 DAI. However, different concentrations (62.5, 125 and 250µg/ml) of nanoSA had stronger effects on PAL activity at 7 DAI. Although, PAL activity did not significantly change in different concentrations of nanosalicylic acid at 11 DAI, demonstrating that high concentration of nanosalicylic acid may have inducible effects on PAL activity at 4 and 7 DAI whereby PAL activity was greatest in wheat roots sprayed with 125µg/ml of nanosalicylic acid at 7 DAI.

In agreement with our findings, there are some reports indicating that peroxidase and PAL are the essential enzymes involved in plant defense against stressors (War *et al.*, 2011 a, b; Usha and Jyothsna, 2010) and that SA has been recognized to stimulate these enzymes in plants (Hu *et al.*, 2009; Lu, 2009; Idrees *et al.*, 2011). Previous study has demonstrated that the applied treatments of SA induce the activities of the PAL enzyme (Danaee *et al.*, 2013). These enzyme activities could be correlated with nematode (*H. avenae*) infection in wheat whereby SA can boost the activity of peroxidase and PAL via reducing nematode penetration, final nematode population and its reproduction (Pokhare *et al.*, 2012; Oka *et al.*, 1997). Recent data have shown that wheat plants treated by SA had a significant increment in hydrogen peroxide and which seems to be related to increased superoxide dismutase and decreased catalase activities which SA may also generate oxidative stress/reactive oxygen species (ROS) in plants (Horváth *et al.*, 2007;

Makandar *et al.*, 2012). In contrast to our findings, there are reports showing that salicylic acid has suppressing effect and can reduce the activity of peroxidase after 6, 10 and 20 days of application (Maksimov *et al.*, 2014). Our results revealed that exposure of wheat leaves to lower concentrations (62.5 and 125 µg/ml) of nano-salicylic acid enhances the peroxidase 113 and 114 genes expression levels at 4 and 7 DAI.

A few studies indicate that three of 13 class III peroxidases are significantly upregulated at 24 h after cereal cyst nematode infection, and their changes in relative expression were more than two-fold (Kong *et al.*, 2015). In addition, There is an analytical research reporting that Genes encoding cytosolic forms of ascorbate peroxidase were induced in roots of both introgression wheat/*Aegilops ventricosa* H-93-8 line, carrying the Cre2 gene, and its parental line H-10-15 as susceptible control in response to nematode infection. (Simonetti *et al.*, 2010).

The induction of the peroxidase activity in susceptible wheat roots could be correlated with physical and chemical barriers such as the cell wall, waxes, hairs, and secondary metabolites against pathogen which typically exhibit increased lignification, an oxidative burst, generation of cytotoxic compounds and induction of defense-related genes (Klotz *et al.*, 1998). Genetic studies indicate that class III peroxidases activity in wheat roots carrying resistance genes may have greater inhibitory effects on cyst nematode infection (Andres *et al.*, 2001; Montes *et al.*, 2004). The induction of PAL activity in susceptible wheat roots could be correlated with production of SA via phenylpropanoid pathway (Wildermuth *et al.*, 2001) and treating with nano-salicylic acid increases the PAL activity. PAL plays a central role in plant defense against invading pathogens such as bacteria, fungi, and viruses, exhibiting increases after infection by nematodes (Cui *et al.*, 2001; Logemann *et al.*, 2000; Vasyukova *et al.*, 2009). The induction in the peroxidase expression level in susceptible wheat roots is dependent on the ROS production level which salicylic acid plays a key role in maintenance of the ROS levels and is necessary for peroxidase activity (Xu *et al.*, 2017). Although, previous research has shown that

systemic increase of H<sub>2</sub>O<sub>2</sub> can increase peroxidase expression levels at 7 DAI in leaves of the susceptible line infected by the *H. avenae* that would occur as a result of lower Ascorbate peroxidase activity in roots of this line (Simonetti *et al.*, 2010). Also several hypotheses have been formulated to explain why the function of salicylic acid in peroxidase expression has not been clearly understood. Our findings indicated that higher nanosalicylic acid concentrations have potential effects on peroxidase and PAL activities in wheat infected by *H. filipjevi* which may be involved in defense mechanisms in plants. We also demonstrated that lower nanosalicylic acid concentrations may induce peroxidase 113-114 expression level in wheat leaves infected by nematode and that high concentration of nano salicylic acid had inhibitory effects on peroxidase gene expression level. In conclusion, the results suggest that SA may play a putative role as a possible signaling component in the case of the infection of plants with nematodes using a model system consisting of wheat.

## References

- Ahmadi, A. R., Tanha Maafi, Z. and Dababat, A. 2013. Crop loss assessment of *Heterodera filipjevi* on some cultivars of wheat, barley and triticale under field condition of Southwest of Iran. Proceeding of The 4<sup>th</sup> International Cereal Nematodes Initiative Workshop. August 22-24, 2013. Beijing, China.
- Ahola-Iivarinen, E., Ronnholm, G. and Somervuo, P. 2009. Quickly-released peroxidase of moss in defense against fungal invaders. *New Phytologist*, 183: 432-43.
- Almaghrabi, O. A., Massoud, S. I. and Abdelmoneim, T. S. 2013. Influence of inoculation with plant growth promoting rhizobacteria (PGPR) on tomato plant growth and nematode reproduction under greenhouse conditions. *Saudi Journal of Biological Sciences*, 20 (1): 57-61.
- Almagro, L., Ros, L. V. G., Belchi-Navarro, S., Bru, R., Barcelo, A. R. and Pedreno, M. A. 2009. Class III peroxidases in plant defence

- reactions. *Journal of Experimental Botany*, 60: 377-90.
- Andres, M. F., Melillo, M. T., Delibes, A., Romero, M. D. and Bleve-Zacheo, T. 2001. Changes in wheat root enzymes correlated with resistance to cereal cyst nematodes. *New Phytologist*, 152: 343-354.
- Anonymous. 2016. *Agricultural Statistical Yearbook*. Tehran: Iran, Ministry of Jihad-E-Agriculture, Statistical and Information Technology Unit.
- Asselbergh, B.O.B., Achuo, A.E., Höfte, M. and Van Gijsegem, F., 2008. Abscisic acid deficiency leads to rapid activation of tomato defence responses upon infection with *Erwinia chrysanthemi*. *Molecular Plant Pathology*, 9 (1), pp. 11-24.
- Bird, D. M., Jones, J. T., Opperman, C. H., Kikuchi, T. and Danchin, E. G. 2015. Signatures of adaptation to plant parasitism in nematode genomes. *Parasitology*, 151: 71-84.
- Chassot, C., Nawrath, C. and Métraux, J. P. 2007. Cuticular defects lead to full immunity to a major plant pathogen. *Plant Journal*, 49: 972-980.
- Cui, Y., Bell, A. A., Joost, O. and Magill, C. 2001. Expression of potential defense response genes in cotton. *Physiological and Molecular Plant Pathology*, 56: 25-31.
- Dababat, A. 2012. *Protocols for Cereal Cyst Nematode (CCN), Root Lesion Nematode (RLN) and Crown Rot (CR) screening implemented by SBP Program CIMMYT-TURKEY*. Soil Borne Pathogens Group, CIMMYT, Turkey.
- Danaee, E., Naderi, R., Kalatejari, S. and Ladan Moghadam, A. R. 2013. Evaluation the effect salicylic acid and benzyladenine on enzymic activities and longevity of gerbera cut flowers. *IRJABS*, 7 (5): 304-308.
- Delibes, A., Andres, M. F. and Lopez-Brana, I. 2009. Analysis of Class III Peroxidase Genes Expressed in Roots of Resistant and Susceptible Wheat Lines Infected by *Heterodera avenae*. *Mol Plant Microbe Interact*, 22: 1081-92.
- Delibes, A., López-Braña, I., Moreno-Vázquez, S., Simonetti, E., Martín- Sánchez, J. A., Sin, E., Martínez, C., Briceño-Félix, G., Michelena, A. and Torres L., Andrés, M. E. and Romero, M. D. 2008. Peroxiadase expression in a cereal cyst nematode (*Heterodera avenae*) resistant hexaploid wheat line. *Annual Wheat Newsletter*, 54: 134-137.
- Hajihassani, A., Tanha Maafi, Z, Nicol, J. M. and Rezaee, S. 2010. Effect of the cereal cyst nematode, *Heterodera filipjevi*, on wheat in microplot trials. *Nematology*, 12 (3): 357-363.
- Hajihassani, M. and Hajihassani, A. 2010. Tolerance limit of winter wheat to cereal cyst nematode, *Heterodera filipjevi*, in pot trials in Iran. *Plant Protection*, 2(1): 69-77.
- Horváth, E., Szalai, G. and Janda, T. 2007. Induction of abiotic stress tolerance by salicylic acid signaling. *Journal of Plant Growth Regulation*, 26: 290-300.
- Hu, X., Li, W., Chen, Q. and Yang, Y. 2009. Early signal transduction linking the synthesis of jasmonic acid in plant. *Plant Signal Behavior*, 4:696-7.
- Idrees, M., Naeem, N., Aftab, T., Masroor, M. and Moinuddin, A. K. 2011. Salicylic acid mitigates salinity stress by improving antioxidant defense system and enhances vincristine and vinblastine alkaloids production in periwinkle. *Acta Physiologiae Plantarum*, 33: 987-99.
- Jing, J., Hewezi, T. and Baum, T. J. 2011. Arabidopsis Peroxidase AtPRX53 Influences Cell Elongation and Susceptibility to *Heterodera schachtii*. *Plant Signal Behavior*, 6 (11): 1778-1786.
- Karimipour Fard, H. Pourjam, E., Tanha Maafi, Z. and Safaie, N. 2018. Assessment of yield loss of wheat cultivars caused by *Heterodera filipjevi* under field conditions. *Journal of Phytopathology*, 166: 299-304. DOI: 10.1111/jph.12686.
- Kaur, K. D., Jha, A., Sabikhi, L. and Singh, A. K. 2014. Significance of coarse cereals in health and nutrition: a review. *Journal of Food Science and Technology*, 51 (8): 1429-1441.
- Ketabchi, S, Majzoob, S. and Charegani, H. A. 2014. Effect of salicylic acid and methyl jasmonate on phenylalanine ammonia-lyase

- activity and total phenol in wheat infected by *Pratylenchus thornei*. Archives of Phytopathology and Plant Protection, 48 (1): 10-17.
- Klotz, K. L., Liu, T. Y., Liu, L. and Lagrimini, L. M. 1998. Expression of the tobacco anionic peroxidase gene is tissue specific and developmentally regulated. Plant Molecular Biology, 36: 509-520.
- Kong, L. A., Wu, D. Q. and Huang, W. K. 2015. Large-scale identification of wheat genes resistant to cereal cyst nematode *Heterodera avenae* using comparative transcriptomic analysis. BMC Genomics, 16: 801.
- Ladner, D. C., Tchounwou, P. B. and Lawrence, G. W. 2008. Evaluation of the Effect of Ecologic on Root Knot Nematode, *Meloidogyne incognita* and Tomato Plant, *Lycopersicon esculenum*. International Journal of Environmental Research and Public Health, 5 (2): 104-110.
- Lareen, A., Burton, F. and Schäfer, P. 2016. Plant root-microbe communication in shaping root microbiomes. Plant Molecular Biology, 90: 575-587.
- Little, D., Gouhier-Darimont, C., Bruessow, F. and Reymond, P. 2007. Oviposition by pierid butterflies triggers defense responses in Arabidopsis. Plant Physiology, 143: 784-800.
- Loake, G. and Grant, M. 2007. Salicylic acid in plant defence-the players and protagonists. Current Opinion in Plant Biology, 10 (5): 466-72.
- Logemann, E., Tavernaro, A., Shulz, W., Somssish, I. E. and Hahlbrock, K. 2000. UV light selectively co induces supply pathways from primary metabolism and flavonoid secondary product formation in parsley. Proceedings of National Academy of Science USA, 97: 1903-1907.
- Lu, H. 2009. Dissection of salicylic acid-mediated defense signaling networks. Plant Signal Behavior, 4: 713-717.
- Makandar, R., Nalam, V. J., Lee, H., Trick, H. N., Dong, Y. and Shah, J. 2012. Salicylic acid regulates basal resistance to Fusarium head blight in wheat. Molecular Plant-Microbe Interactions, 25 (3): 431-439.
- Maksimov, I. V., Cherepanova, E. A., Surina, O. B. and Sakhabutdinova, A. R. 2014. The Effect of Salicylic Acid on Peroxidase Activity in Wheat Calli Cocultured with the Bunt Pathogen *Tilletia caries*. Journal of Plant Interactions, 9 (1): 306-314.
- Malik, C.P. and Singh, M.B., 1980. Plant enzymology and histo-enzymology.
- Mesgaran, M.B., Madani, K., Hashemi, H. and Azadi, P., 2017. Iran's land suitability for agriculture. Scientific Reports, 7 (1): 7670.
- Montes, M. J., Lopez-Brana, I. and Delibes, A. 2004. Root enzymeactivities associated with resistance to *Heterodera avenae* conferred gene by Cre7 in a wheat/*Aegilops triuncialis* introgression line. Journal of Plant Physiology, 161: 1135-1140.
- Oka, Y. and Cheon, Y. 2001. Induced Resistance to Cyst and Root-knot Nematodes in Cereals by DL- $\beta$ -amino-n-butyric Acid. EJPP, 107: 219
- Oka, Y., Chet, I. and Spiegel, Y. 1997. An immunoreactive protein to wheat-germ agglutinin antibody is induced in oat roots following invasion of the cereal cyst nematode *Heterodera avenae*, and by jasmonate. MPMI, 10: 961-969.
- Peltonen, S., and Karjalainen, R. 1995. Phenylalanine ammonialyase activity in barley after infection with *Bipolaris sorokiniana* or treatment with its purified xylanase. Journal of Phytopathology, 143 (4), 239-245.
- Periago, P. M., Barcelo, A. R. and Martinez-Gomez, P. 2006. The apoplastic antioxidant system in Prunus: response to long-term plum pox virus infection. Journal of Experimental Botany, 57: 3813-24.
- Pokhare, S., Pankaj, Shakil, N. A., Kumar, J. and Singh, K. 2012. Foliar application of chemical elicitors induces biochemical changes in wheat against the cereal cyst nematode, *Heterodera avenae*. Nematologia, 40: 181-187.
- Rabe, F., Ajami-Rashidi, Z., Doehlemann, G., Kahmann, R. and Djamei, A. 2013.

- Degradation of the plant defence hormone salicylic acid by the biotrophic fungus *Ustilago maydis* *Molecular Microbiology*, 89 (1): 179-88.
- Rejeb, I.B., Pastor, V. and Mauch-Mani, B., 2014. Plant responses to simultaneous biotic and abiotic stress: molecular mechanisms. *Plants*, 3 (4), pp.458-475.
- Riley, I. T., Nicol, J. M. and Dababat, A. A. 2009. Cereal Cyst Nematodes: Status, Research and Outlook. CIMMYT, Ankara, Turkey.
- Simonetti, E., Alba, E., Montes, M. J., Delibes, A. and López-Braña, I. 2010. Analysis of ascorbate peroxidase genes expressed in resistant and susceptible wheat lines infected by the cereal cyst nematode, *Heterodera avenae*. *Plant Cell Reports*, 29 (10): 1169-78.
- Smiley, R. W., Dababat, A. A., Iqbal, S., Jones, M. G. K., Tanha Maafi, Z., Peng, D., Subbotin, S. A., and Waeyenberge, L. 2017. Cereal cyst nematodes: A complex and destructive group of *Heterodera* species. *Plant Disease*, 101: 1692-1720.
- Timper, P. 2014. Conserving and Enhancing Biological Control of Nematodes. *Journal of Nematology*, 46 (2): 75-89.
- Usha Rani, P. and Jyothsna, Y. 2010. Biochemical and enzymatic changes in rice as a mechanism of defense. *Acta Physiologiae Plantarum*, 32:695-701.
- Van Loon, L. C., Rep, M. and Pieterse, C. M. 2006. Significance of inducible defense-related proteins in infected plants. *Annual Review of Phytopathology*, 44: 135-162.
- Vasyukova, N. I. and Zinovieva, S. V., Udalova, Zh. V., Gerasimova, N. G., Ozeretskovskaya, O. L. and Sonin, M. D. 2009. Jasmonic acid and tomato resistance to the root-knot nematode, *Meloidogyne incognita*. *Doklady Biological Sciences*, 428: 448-450.
- War, A. R., Paulraj, M. G., Ahmad, T., Buhroo, A. A., Hussain, B. and Ignacimuthu S. 2012. Mechanisms of plant defense against insect herbivores. *Plant Signal Behavior*, 7 (10): 1306-1320.
- War, A. R., Paulraj, M. G., War, M. Y. and Ignacimuthu, S. 2011 a. Jasmonic acid-mediated induced resistance in groundnut (*Arachis hypogaea* L.) against *Helicoverpa armigera* (Hubner) (Lepidoptera: Noctuidae). *Journal of Plant Growth Regulation*, 30: 512-523.
- War. A. R., Paulraj, M. G., War, M. Y. and Ignacimuthu, S. 2011 b. Role of salicylic acid in induction of plant defense system in chickpea (*Cicer arietinum* L). *Plant Signal Behavior*, 6 (11): 1787-1792.
- Wildermuth, M. C., and Dewdney, J., Wu, G. and Ausubel, F. M. 2001. Isochorismate synthase is required to synthesize salicylic acid for plant defence. *Nature*, 414: 562-565.
- Xu, L., Zhao, H., Ruan, W., Deng, M., Wang, F., Peng, J. 2017. Abnormal inflorescence meristem1 functions in salicylic acid biosynthesis to maintain proper reactive oxygen species levels for root meristem activity in rice. *Plant Cell*, 29 (3): 560-574.
- Zhang, L., Lilley, C. J., Imren, M., Knox, J. P., and Urwin, P. E. 2017. The Complex Cell Wall Composition of Syncytia Induced by Plant Parasitic Cyst Nematodes Reflects Both Function and Host Plant. *Front Plant Sci*, 8: 1087.
- Zinovieva, S. V. 2014. Co-adaptation mechanisms in plant-nematode systems. *Parazitologiya*, 48 (2): 110-30.

## تأثیر فرمولاسیون نانوسالیسیلیک اسید بر بیان ژن‌های پراکسیداز (۱۱۳-۱۱۴) و پراکسیداز و فنیل آلانین آمونیا لایز در گندم رقم حساس به *Heterodera filipjevi*

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**چکیده:** اثر فرمولاسیون نانوسالیسیلیک اسید بر بیان ژن‌های پراکسیداز (۱۱۳-۱۱۴) و آنزیم‌های پراکسیداز و فنیل آلانین آمونیا لایز (PAL) در گیاهان گندم رقم حساس به *Heterodera filipjevi* مورد بررسی قرار گرفت. ریشه و برگ گندم B ezostaya به‌طور تصادفی به گروه شاهد و گروه‌های در معرض به ترتیب با ۶۲،۵، ۱۲۵ و ۲۵۰ میکروگرم در میلی لیتر نانوسالیسیلیک اسید تقسیم شدند. آنالیز اسپکتروفتومتری عصاره‌های ریشه‌ی گیاهان آلوده، ۴، ۷ و ۱۱ روز پس از تلقیح نماتد (DAI) برای ارزیابی آنزیم‌های پراکسیداز و PAL و آنالیز بیان ژن‌های پراکسیداز (۱۱۳-۱۱۴) با استفاده از Realtime-PCR انجام شد. فعالیت پراکسیداز به‌طور معنی‌داری در تیمارهای تحت تأثیر ۲۵۰ میکروگرم در میلی لیتر نانوسالیسیلیک اسید پس از ۱۱ روز افزایش داشت. فعالیت PAL تحت تأثیر ۲۵۰ و ۱۲۵ میکروگرم در میلی لیتر نانوسالیسیلیک اسید در مقایسه با کنترل در چهار و هفت DAI افزایش یافت. فعالیت PAL نیز در نمونه‌های در معرض ۶۲،۵ و ۲۵۰ میکروگرم در میلی لیتر نانوسالیسیلیک اسید نسبت به کنترل در هفت DAI تشدید شد. دوز ۶۲،۵ میکروگرم در میلی لیتر نانوسالیسیلیک اسید باعث افزایش سطح بیان پراکسیداز ۱۱۳-۱۱۴ در برگ گندم در چهار DAI و هم‌چنین افزایش سطح بیان ژن‌های پراکسیداز ۱۱۳-۱۱۴ در سطح ۱۲۵ و ۲۵۰ میکروگرم در میلی لیتر در مقایسه با کنترل در چهار و هفت DAI شد. بین بیان ژن‌ها در معرض ۶۲،۵، ۱۲۵ و ۲۵۰ میکروگرم در میلی لیتر نانوسالیسیلیک اسید در مقایسه با کنترل در یازده DAI کاهش معنی‌داری وجود داشت. نتایج نشان داد که غلظت‌های بالای نانوسالیسیلیک اسید تأثیر بالقوه‌ای بر فعالیت آنزیم‌های پراکسیداز و PAL در گندم آلوده به *H. filipjevi* دارد و غلظت بالای نانوسالیسیلیک اسید اثر مهاری بر سطح بیان ژن پراکسیداز دارا می‌باشد.

**واژگان کلیدی:** نماتد سیستی غلات، گندم، پراکسیداز، سالیسیلیک اسید